

RTS,S Malaria Vaccine and Increased Mortality in Girls

Sabra L. Klein,^a Frank Shann,^b William J. Moss,^c Christine S. Benn,^d Peter Aaby^e

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA^a; Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia^b; Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA^c; Research Centre for Vitamins and Vaccines, Bandim Health Project, Statens Serum Institute, Copenhagen, Denmark^d; Bandim Health Project, InDEPTH Network, Bissau, Guinea-Bissau^e

Malaria was estimated to result in 214 million clinical cases and 438,000 deaths in 2015, primarily in children under 5 years of age. In Africa, malaria causes approximately 10% of all deaths in children under 5 years of age. The RTS,S/AS01 malaria vaccine has been tested in young children in phase III clinical trials and shown to be 18 to 36% efficacious against clinical malaria (1). Although the vaccine may be efficacious against clinical malaria, it does not however reduce overall mortality.

The World Health Organization (WHO) recently published a position paper on malaria vaccines (2), with emphasis on the RTS,S/AS01 vaccine. Although the vaccine has had modest efficacy, the data in Table 1 show that RTS,S was associated with higher all-cause mortality in girls (mortality ratio, 1.91; 95% confidence interval [CI], 1.30 to 2.79; $P = 0.0006$) but not in boys (mortality ratio, 0.84; 95% CI, 0.61 to 1.17; $P = 0.3343$) in both age groups in which the vaccine was tested (i.e., 6 to 12 weeks and 5 to 17 months) (<http://www.gsk-clinicalstudyregister.com/files/2/9a7b7726-34e2-418d-bea6-c3fb071fd51c>). The sex-differential effect is highly significant ($P = 0.001$). There also was a tendency for RTS,S to be associated with a higher risk of fatal malaria in girls (malaria mortality ratio, 1.90 [0.82 to 4.37]) but not in boys (malaria mortality ratio, 1.07 [0.52 to 2.18]). It is counterintuitive that there was no reduction in fatal malaria associated with RTS,S; however, RTS,S was associated with a twofold-higher case fatality ratio in children who got severe malaria (3).

The WHO has speculated that the increased mortality in girls was “largely due to the low female mortality in the control arm” and “could be due to chance” (2), despite the P value of 0.0006 for girls and a mortality rate after RTS,S of 2.4% in girls compared to 1.8% in boys (risk ratio, 1.33 [1.02 to 1.74]) (Table 2). Although

the WHO could be correct in speculating that this finding was due to chance, these numbers suggest a need for caution and additional research. Before RTS,S is introduced into routine vaccination schedules, we should determine whether RTS,S/AS01 increases mortality in girls and investigate possible mechanisms.

There is precedent for the observation that infant girls experience increased mortality following receipt of vaccines. For example, in the 1980s, when the high-titer measles vaccine (HTMV) was introduced to prevent measles in children under 9 months of age, there was a twofold increase in all-cause mortality in girls, but no increase in boys, which led to withdrawal of the vaccine (4). It was subsequently determined that the increased mortality occurred only among girls who received diphtheria-tetanus-pertussis (DTP) vaccine after HTMV and not among girls who received HTMV after their last dose of DTP (5). The interaction between HTMV and DTP may have caused nonspecific negative effects on all-cause mortality in girls but not boys. Evidence from multiple studies of nonlive vaccines, including DTP and the inactivated polio vaccine (IPV), show that these nonlive vaccines have greater detrimental effects for girls than boys (5, 6). Therefore, the increased female mortality after RTS,S/AS01 should not be dismissed as an unexpected finding that occurred by chance. Further clinical studies should explore whether

Published 26 April 2016

Citation Klein SL, Shann F, Moss WJ, Benn CS, Aaby P. 2016. RTS,S malaria vaccine and increased mortality in girls. *mBio* 7(2):e00514-16. doi:10.1128/mBio.00514-16.

Copyright © 2016 Klein et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Sabra L. Klein, sklein2@jhu.edu.

TABLE 1 RTS,S malaria vaccine and mortality by sex

	No. of deaths overall [no. of deaths due to malaria]/no. of persons in group (%)				
Sex and age of group	R3R ^a	R3C ^b	R3R and R3C groups combined	C3C ^c	RTS,S recipient/control risk ratio (95% CI)
Males					
5–17 mo	26 [4]/1,509 (1.7)	19 [9]/1,472 (1.3)	45 [13]/2,981 (1.5)	29 [8]/1,471 (2.0)	0.77 (0.48–1.22)
6–12 wk	24 [3]/1,116 (2.2)	26 [8]/1,118 (2.3)	50 [11]/2,234 (2.2)	26 [3]/1,079 (2.4)	0.93 (0.58–1.48)
Total			95 [24]/5,215 (1.8)	55 [11]/2,550 (2.2)	0.84 (0.61–1.17)
Females					
5–17 mo	35 [9]/1,467 (2.4)	32 [8]/1,500 (2.1)	67 [17]/2,967 (2.3)	17 [4]/1,503 (1.1)	2.00 (1.18–3.39)
6–12 wk	27 [5]/1,064 (2.5)	29 [4]/1,060 (2.7)	56 [9]/2,124 (2.6)	16 [3]/1,100 (1.5)	1.81 (1.04–3.14)
Total			123 [26]/5,091 (2.4)	33 [7]/2,603 (1.3)	1.91 (1.30–2.79)

^a R3R, 3× RTS,S plus booster RTS,S.

^b R3C, 3× RTS,S plus comparator vaccine.

^c C3C, controls (comparator vaccines).

TABLE 2 Female-male mortality risk ratio in RTS,S malaria vaccine recipients

Age group	Female/male risk ratio (95% CI)		
	R3R ^a	R3C ^b	R3R and R3C combined
5–17 mo	1.38 (0.84–2.29)	1.65 (0.94–2.90)	1.50 (1.03–2.18)
6–12 wk	1.18 (0.69–2.03)	1.18 (0.70–1.98)	1.18 (0.81–1.72)
Total			1.33 (1.02–1.74)

^a R3R, 3× RTS,S plus booster RTS,S.^b R3C, 3× RTS,S plus comparator vaccine.

girls need lower doses of the RTS,S/AS01 vaccine or should receive the vaccine with or separately from other vaccines or at different ages than boys.

Preclinical studies in animal models can help provide insights into the biological basis of these observations, but here too, analysis of potential sex effects has been lacking. Published studies of RTS,S or recombinant circumsporozoite protein in mice and non-human primates have only reported using adult females or have not reported the sex of the animals (7–9). Generally, in the fields of immunology, vaccinology, and infectious diseases, investigators either do not report the sex of their animals or predominately use female animals (10). This “one size fits all” approach to vaccine research is not working. Preclinical studies should consider how both age and sex affect vaccine responses and outcomes. RTS,S vaccine could also be used to uncover immunological mechanisms for a possible increase in mortality after RTS,S vaccination among girls but not boys.

The RTS,S vaccine is modestly effective at reducing clinical malaria in children, but the sex differences in all-cause mortality should be rigorously studied in both clinical trials and experimental animal models, particularly in light of prior experience with the HTMV. We seek to raise awareness about the need for additional research into how the RTS,S vaccine and, possibly, other vaccines are associated with greater mortality in girls but not boys. This will only be achieved if age and sex are considered in *a priori* hypotheses in vaccine trials to identify and address potential risks early in the vaccine development process.

ACKNOWLEDGMENTS

The work on the nonspecific effects of vaccines was supported by European Union FP7 support for OPTIMUNISE (grant number Health F3-

2011–261375) and the Danish National Research Foundation (grant number DNRFI08).

REFERENCES

1. RTS/S Clinical Trials Partnership. 2015. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 386:31–45. [http://dx.doi.org/10.1016/S0140-6736\(15\)60721-8](http://dx.doi.org/10.1016/S0140-6736(15)60721-8).
2. WHO. 2016. Malaria vaccine: WHO position paper-January 2016. *Wkly Epidemiol Rec* 91:33–52.
3. Aaby P, Rodrigues A, Kofoed P-E, Benn CS. 2015. RTS,S/AS01 malaria vaccine and child mortality. *Lancet* 366:1735–1736.
4. Aaby P, Knudsen K, Whittle H, Lisse IM, Thaarup J, Poulsen A, Sodemann M, Jakobsen M, Brink L, Gansted U. 1993. Long-term survival after Edmonston-Zagreb measles vaccination in Guinea-Bissau: increased female mortality rate. *J Pediatr* 122:904–908. [http://dx.doi.org/10.1016/S0022-3476\(09\)90015-4](http://dx.doi.org/10.1016/S0022-3476(09)90015-4).
5. Aaby P, Jensen H, Samb B, Cisse B, Sodemann M, Jakobsen M, Poulsen A, Rodrigues A, Lisse IM, Simondon F, Whittle H. 2003. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. *Lancet* 361: 2183–2188. [http://dx.doi.org/10.1016/S0140-6736\(03\)13771-3](http://dx.doi.org/10.1016/S0140-6736(03)13771-3).
6. Aaby P, Garly ML, Nielsen J, Ravn H, Martins C, Balé C, Rodrigues A, Benn CS, Lisse IM. 2007. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: observations from vaccination trials in Guinea-Bissau. *Pediatr Infect Dis J* 26:247–252. <http://dx.doi.org/10.1097/01.inf.0000256735.05098.01>.
7. Noe AR, Espinosa D, Li X, Coelho-Dos-Reis JG, Funakoshi R, Giardina S, Jin H, Retallack DM, Haverstock R, Allen JR, Vedvick TS, Fox CB, Reed SG, Ayala R, Roberts B, Winram SB, Sacchi J, Tsuji M, Zavala F, Gutierrez GM. 2014. A full-length Plasmodium falciparum recombinant circumsporozoite protein expressed by Pseudomonas fluorescens platform as a malaria vaccine candidate. *PLoS One* 9:e107764. <http://dx.doi.org/10.1371/journal.pone.0107764>.
8. Mettens P, Dubois PM, Demoitie MA, Bayat B, Donner MN, Bourguignon P, Stewart VA, Heppner DG, Garçon N, Cohen J. 2008. Improved T cell responses to Plasmodium falciparum circumsporozoite protein in mice and monkeys induced by a novel formulation of RTS,S vaccine antigen. *Vaccine* 26:1072–1082. <http://dx.doi.org/10.1016/j.vaccine.2007.12.018>.
9. Pichyangkul S, Kum-Arb U, Yongvanitchit K, Limsalaketch A, Gettayacamin M, Lanar DE, Ware LA, Stewart VA, Heppner DG, Mettens P, Cohen JD, Ballou WR, Fukuda MM. 2008. Preclinical evaluation of the safety and immunogenicity of a vaccine consisting of Plasmodium falciparum liver-stage antigen 1 with adjuvant AS01B administered alone or concurrently with the RTS,S-AS01B vaccine in rhesus primates. *Infect Immun* 76:229–238. <http://dx.doi.org/10.1128/IAI.00977-07>.
10. Beery AK, Zucker I. 2011. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* 35:565–572. <http://dx.doi.org/10.1016/j.neubiorev.2010.07.002>.

The views expressed in this Guest Editorial do not necessarily reflect the views of this journal or of ASM.